

Syahidah_2019_IOP_Conf._Ser. __Earth_Environ._Sci._270_012 051.pdf

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Submission date: 05-Jul-2020 08:51AM (UTC+0700)

Submission ID: 1353497030

File name: Syahidah_2019_IOP_Conf._Ser.__Earth_Environ._Sci._270_012051.pdf (450.32K)

Word count: 3152

Character count: 17602

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To cite this article: Syahidah and N Subekti 2019 *IOP Conf. Ser.: Earth Environ. Sci.* **270** 012051

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Biological Activity of Mangrove Leaves Extract (*Rhizophora* sp.)

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Abstract. Several of mangrove species have very large applications in the traditional folk medicine; various parts of these plants are used by the local people as a cure for the various illness. Anti-termite and antifungal activity of mangrove leaves were investigated. The leaves mill was extracted with acetone and methanol to give their extracts, each of which were fractionated successively using *n*-hexane, ethyl acetate, and water to afford their fractions. The yield of the extracts and fractions suggested that the extractive of mangrove leaves tend to be polar. It was demonstrated that the methanol extract itself, the ethyl acetate fraction and the *n*-hexane fraction from the methanol extract, and the ethyl acetate fraction from the acetone extract had high antifeedant activity against *Coptotermes formosanus*. On the other hand, the extract of mangrove leaves indicated the higher activities against *Trametes Versicolor* compared to the *Fomitopsis palustris* fungi.

1. Introduction

In tropical regions, mangroves play an important role for environmental services, economy, and social benefits. Mangroves contribute to numerous environmental services, including trapping and recycling organic matter, providing shelters and surfaces for terrestrial and aquatic organisms, and contributing to the overall health of coastal environments. The mangrove ecosystem represents an interphase between terrestrial and marine communities, which receive a daily input of water from the ocean (tides) and freshwater, sediments, nutrients and silt deposits from upland rivers. Mangroves may grow as trees or shrubs according to the climate, salinity of water, topography and edaphic features of the area in which they exist. Mangroves are salt-tolerant evergreen forests found along sheltered coastlines, shallow-water lagoons, estuaries, rivers or deltas in 124 tropical and subtropical countries and areas [1,2].

Mangroves also are well known for their ecological importance and a rich source of several bioactive compounds such as steroids, triterpenes, saponins, flavonoids, alkaloids and tannins having therapeutic significance. These plants have been extensively used in traditional medicine, and different studies have revealed their activity against human, animal and plant pathogens. The leaf and stem extracts of *S. maritima* are a rich sources of natural antioxidant with moderate antimicrobial activities. It has also been reported that these are an excellent source of antiviral compounds as compared to the seaweeds and seagrasses [3,4].



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In the present study, an attempt was made to evaluate the anti-termite and antifungal activities of *Rhizophora* sp. with a view to evaluate the efficiency of the plant for its possible biopesticides applications. Termites are the greatest importance in recycling woody and other plant material. They can decompose cellulose, the main component of wood. They are abundant in tropical and subtropical environments, where they help in breaking down and recycling one-third of the annual production of dead wood as well as in the recycling of dung of herbivores. Their tunneling efforts help to aerate the soil. On the other hand, termites become economic pests when they start destroying wood and wooden products of human homes, building materials, forests, and other commercial products. They also are the most problematic pest threatening agriculture and the urban environment [5,6].

Moreover, fungi play various important roles in the forest ecosystem, including ectomycorrhizal fungi, arbuscular mycorrhizal fungi, saprotrophic or wood-decomposing fungi, plant pathogenic fungi, and bio-remedial fungi. The fungi were contributed significantly to carbon recycling, as the residues remaining from the harvest of trees and logs are attacked and degraded, particularly by white and brown rot fungi which are more aggressive colonizers and degraders of wood in such environments. Wood-decaying fungi lead to great economic losses of lignocellulosic materials. Several types of fungal wood decay are recognized including white-rot, brown-rot, soft-rot, staining fungi, and molds [7-10].

2. Material and Methods

2.1. Mangrove leaves (*Rhizophora* sp.)

Mangrove fresh and mature leaves were collected from the mangrove forest in Semarang, Central Java Province, Indonesia.

2.2. Extraction and fractionation

The leaves of the plant were dried and then grounded in a hammer mill. Then the leaves mill (40-60 mesh) extracted according to the procedure reported previously with slight modification (Wang et al., 2005). In this study, leaves mill was used instead of small wood-pieces and the leaves mill was extracted with acetone first and the residue was then extracted again using methanol until the extract solution became colorless. These acetone and methanol extracts were then successively fractionated into n-hexane, ethyl acetate, and water to give their soluble fractions.

2.3. Termite test

The termites were obtained from Forestry and Forest Products Research Institute, Japan.

2.4. Fungal strains

The fungal strains used were a white rot fungus (*Trametes versicolor*, NBRC 4937) and brown rot fungus (*Fomitopsis palustris*, NBRC 30339) that were purchased from Biological Resource Center, National Institute of Technology and Evaluation, Japan.

2.5. Termite bioassay

No-choice and two-choice tests were employed to assess the termiticidal activity of mangrove leaves [11]. The weight loss of the paper discs were used to determine the termiticidal properties of extract that was obtained by the following equation: in the no-choice bioassay, the absolute coefficient of antifeedancy (A) = $[(KK - EE)/(KK + EE)] \times 100(\%)$, while in the two-choice bioassay, the relative coefficient of antifeedancy (R) = $[(K - E)/(K + E)] \times 100(\%)$; where KK (K) and EE (E) are the weight losses of the control and treated paper discs, respectively. The total coefficient of antifeedancy (T) is equal to A plus R. All extracts tested were classified into the following classes according to their T values; feeding preference (T < 0), class I (0 ≤ T < 50), class II (50 ≤ T < 100), class III (100 ≤ T < 150), class IV (150 ≤ T < 200) and 200 for complete antifeedant.

2.6. Fungal bioassay

The fungal bioassay was conducted using the Potato dextrose agar (PDA) [12]. PDA powder was added into distilled water (39g/L), after sterilizing, it was mixed with 50 and 100 ppm of extracts test in a Petri

dish, and the mycelium disk was placed at the center of the medium. The Petri dishes were incubated at 23°C. When the mycelium of fungi reached the edges of the control Petri dish, the antifungal index (%) was calculated as follows: antifungal index (%) = $(1 - Da/Db) \times 100$, where Da: diameter of mycelium colony growth with sample (cm), Db: diameter of mycelium colony growth in control (cm). PDA plates containing methanol without sample solutions were used as a control. Based on antifungal activity (AFA) value, the activity of each fraction was then classified into activity category level. $AFA \geq 75\%$ (very strong), $75\% \leq AFA < 50\%$ (strong), $50\% \leq AFA < 25\%$ (moderate), $25\% \leq AFA < 0$ (weak) and 0 (not active).

2.7. Statistical analyses

The SPSS software was utilized as a statistical tool. As a result of multiple analyses of variation (ANOVA) tests, the type of extracts of all the samples was evaluated their anti termite and antifungal activity. For Post Hoc analyses, a Scheffe test was used to compare values at a level of significance of $p < 0.05$.

3. Results and discussion

3.1 Extract yield

Extraction and fractionation of samples showed that the methanol extract (10%) had a higher percentage than the acetone extract (5.20%), and the successive fractionation of acetone extract resulted in the obtained fractions namely, *n*-hexane (39.50%), ethyl acetate (59.18%) and aqueous (1.87%), respectively. Moreover, the methanol extracts resulted in *n*-hexane (2.97%), ethyl acetate (4.36%) and aqueous (84.35%), respectively. This data indicated that the extracts of mangrove leave would tend to be polar.

The amount and composition of plant extractives depends on several factors such as plant species, age, and growth location. The accumulation of secondary metabolites highly depends on temperature and light intensity conditions and phenological cycle. Plants of the same species occurring in different environments may differ significantly in their content of particular secondary metabolites [13]. The phenolic compound quantities changed greatly during plant development and several studies have been reported that the phenolic compound of extractive also has many activities such as antioxidant, anti-inflammatory, antibacterial and antifungal action against causal agents of human intestinal and respiratory tract infections and fungicidal properties thus protects the tree against microbiological attack [14–17].

3.2 Antitermite activity

3.2.1 Weight loss

Weight loss of the paper discs data was displayed in Figure 1 and 2, representing the protection ability of the extracts and their fractions against *C. formosanus*. No-choice bioassay method for testing termiticidal activity, while two-choice bioassay method for testing antifeedant activity.

The data suggested that there are significant differences on the protection of these extracts against termite, where the *n*-hexane fraction of methanol extract from no-choice bioassay generated the lowest weight loss of paper discs compare to the other fractions (2.4%).

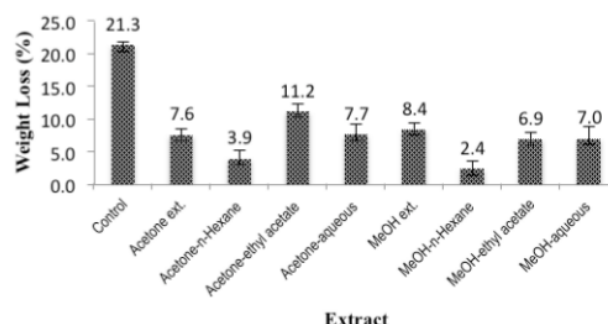


Figure 1. Weight loss of paper discs in no-choice bioassay

As shown in Figure 1 above, the weight loss of all the extracts and fractions lower than the control. This results confirmed that the extracts and fractions have termiticidal activity, which indicated these extracts and fractions were able to deliver reliable protection against *C. formosanus*.

Furthermore, weight loss of treated discs mostly lower than control in two-choice bioassay as shown in Figure 2. This finding data indicated that these extracts and fractions test had antifeedant activity.

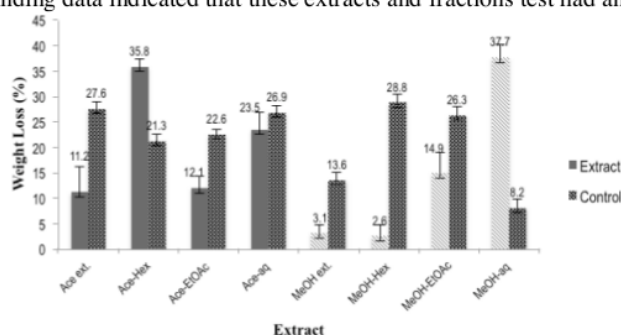


Figure 2. Weight loss of paper discs in two-choice bioassay

3.2.2 Antifeedant activity

Termite bioassay results as shown in Figure 3 exhibited that the methanol extract itself and its n-hexane and ethyl acetate fractions indicated the higher anti termite activity compared to the acetone extract and its fractions. Based on the antifeedancy classification, the methanol extract and ethyl acetate fraction were classified into class III, while the n-hexane fraction revealed the highest activity that is including into class IV. On the contrary, the aqueous fraction of methanol extract and n-hexane fraction of acetone extract exhibited feeding preference activity which means that the Formosan termites preferred to consume the filter paper containing those extracts.

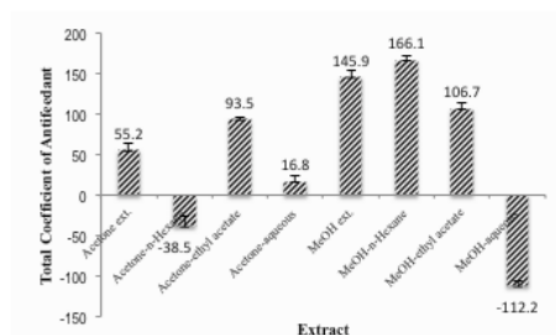


Figure 3. Total Coefficient of Antifeedant of mangrove leaves

The fraction that indicated feeding preference properties might contain substances, such as sugar, vanillin and something else that are digestible and preferable by termite. On the other hand, the resistance to termite attack is due to the presence of some active components of wood as parts of their natural defense, such as flavonoids that possess natural repellent having both toxicity and antioxidant properties. Extractives present in these extract could be toxic or unfavorable for termites, where the ethyl acetate extract was responsible for the greater resistance of heartwood in teak wood [18]. Some flavonoids, such as quercetin and taxifolin might be useful for termite control agents because they are abundant in plants.

3.3 Antifungal activity

The n-hexane fraction of methanol extract of mangrove leaves possesses strong antifungal activity towards *T. versicolor*, while the ethyl acetate and aqueous fractions belonging into the moderate activity against *T. versicolor*. Otherwise, the methanol extract revealed weak activity. Moreover, the acetone extract, ethyl acetate and aqueous fractions of the acetone extract indicated the moderate activity against *T. versicolor*, while the n-hexane fraction showed the weak activity.

On the contrary, both of the acetone and methanol extracts and all their fractions performed weak activity against *F. palustris*.

The strong activity means that the extract has strong inhibition to fungal growth. Presumably, the extract might contain substances that in fact supported the life of the fungi or might contain substances that are digestible and preferable by the fungi [10]. Liriodenine, an alkaloid, that was isolated and identified from n-hexane fraction of *Michelia formosana* could effectively inhibit the growth of wood-rotting fungi [19].

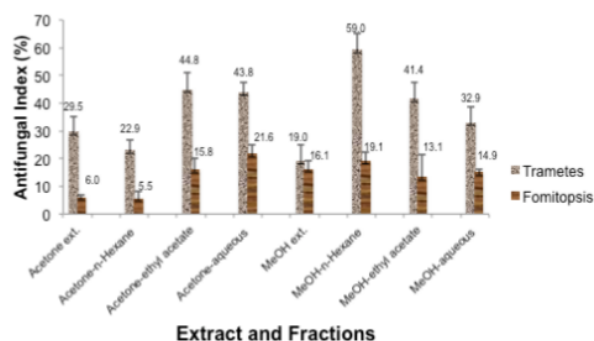


Figure 4. Antifungal index of mangrove leaves against wood-rotting fungi

Phytochemicals such as terpenoids, alkaloids, condensed tannins, and many others consist of extractives play an important role in natural durability. Hardwood species commonly vulnerable to be attacked by white rot fungus. On the contrary, softwood species are commonly nondurable against brown rot fungus.

Cinnamaldehyde, a major constituent of cinnamon essential oils, occurs naturally in the bark and leaves of cinnamon trees of the genus *Cinnamomum*. It has been proven to have strong antifungal activities against a wide variety of wood decay fungi and is a potential candidate for effective and environmentally-benign wood preservatives. Furthermore, eugenol has been demonstrated as an excellent fungicide against wood decay fungi. Moreover, the high antifungal activity of mimosa and quebracho extracts are believed to be related to their high tannin contents. T-murolol and α -cardinal possess antifungal activities against a broad spectrum of tested plant pathogenic fungi and could be used as potential antifungal agents [12,20–22].

4. Conclusion

The Extraction and fractionation of mangrove leaves indicated that the extracts tend to be polar. According to anti termite test, the mangrove leaves heartwood revealed the high antifeedant activity against the subterranean termite *C. formosanus*. Furthermore, the mangrove leaves heartwood exhibited the moderate-strong antifungal ability against *T. versicolor*, and conversely, almost these extracts and fractions showed the weak activity against *F. palustris*.

Acknowledgments

The authors are grateful to Ms. Dewi Primayanti for her assistance in work.

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